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Remarks:
This application was filed on 26 - 11 - 2001 as a divisional application to the application mentioned under INID code 62.

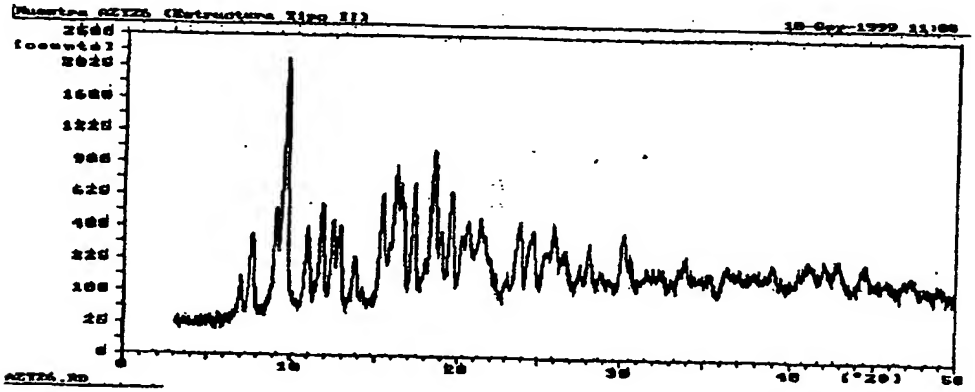
(54) Preparation of crystalline azithromycin dihydrate.

(57) The present invention describes a new procedure for the preparation of the macrolide azithromycin in its crystalline dihydrate form, which is characterized and clearly differentiated by means of the following methods and techniques:

1. IR Spectroscopy.
2. Differential Scan Calorimetry (DSC).
3. X-Ray Diffraction.

Figure 4

Crystalline Azithromycin dihydrate



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Description

BACKGROUND OF THE INVENTION

1. Field of the Invention.

[0001] Azithromycin is the USAN generic name of the azalide 9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin A, which systematic name is 1-oxa-6-azacyclopentadecan-15-one, 13-((2,6-dideoxy-3-C-methyl-1-3-O-methyl-alpha-L-ribo-hexopyranosyl)-oxy)-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-((3,4,6-trideoxy-3-(dimethyl-amino)-beta-D-xylo-hexopyranosyl)oxy). It is a semisynthetic macrolide that shows an excellent antimicrobial activity against gram-positive and some cases of gram-negative bacteria (H.A. Kirst, G.D. Sides, *Antimicrob. Agents. Chemother.* **1989,** 33, 1419-1422). Clinical use of this macrolide is broadening its application to the treatment of opportunistic infections (F. Lecomte, *Rev. Med. Interne* **1998**, *19(4)*, 255-61; S. Alvarez-Elcoro, *Mayo Clin. Proc.* **1999**, *74(6)*, 613-34; J. Schater, *Lancet,* **1999,** 354(9179), 630-35).

2. Description of the Prior Art.

[0002] **Figure 1** shows the different synthetic routes to azithromycin **1**. The names of the intermediates displayed in **Figure 1** are gathered in the following table.

Intermediate	Name
1	Azithromycin
2	Erythromycin A oxime
3	6,9-iminoether
4	9,11-iminoether
5	Azaerythromycin A
6	Azaerythromycin 11,12-hydrogenorthoborate
7	Azithromycin 11,12-hydrogenorthoborate

[0003] The following table summarizes the patents, articles, authors and applicants that describe the different synthetic paths **(A, B, C, D, E)** towards azithromycin **1**.

Route	Patents	Articles	Author	Applicant
A	a) US 4,328,334 • US 4,517,359	• *J. Chem.* Sac. *Perkin Trans 1*, **1986,** 1881 • *J. Chem. Res.*, **1988**, 132 • *Idem miniprint.*, 1988, 1239	S. Djokic	PLIVA
B	b) US 4,474,768		G.M. Bright	PFIZER
C	c) US 5,686,587 d) EP 0,699,207 e) ES 2,104,386		B.V. Yang	PFIZER
D	f) US 5,869,629 g) EP 0,827,965 h) ES 2,122,905	• *J.Org.Chem*, **1997**, *62, (21),* 7479-7481 • *Magn. Reson Chem*, **1998**, 36, 217-225	M. Bayod	ASTUR PHARMA
E	i) EP 0,879,823		W. Heggie	HOVIONE

[0004] The structural elucidation studies carried out with azithromycin **1** have shown the existence of two different crystalline forms: hygroscopic monohydrate and non-hygroscopic dihydrate, being the latter preferred for manufacturing formulations used in therapeutical treatments, as it is described in **EP 0,298,650**.

Azithromycin dihydrate is easily distinguishable from hygroscopic azithromycin by means of the following differentiative assays:

a) The dihydrate form keeps its percentile water content constant at values (4.5-5%) which are very close to the

theoretical value (4.6%).

b) The differential calorimetry analysis (DSC) of azithromycin dihydrate reveals the presence of a single endotherm which may vary between 115 and 135 °C, with an energy absorbed during the process which ranges from 27 to 34 cal/g.

c) Each crystalline form presents its own characteristic X-Ray Diffraction spectrum.

d) The infrared spectra in KBr of both crystalline forms present clear differences:

azithromycin dihydrate	azithromycin monohydrate
$\nu(cm^{-1})$	$\nu(cm^{-1})$
3560 and 3496 (2 sharp bands)	3500 (wide band)
1344	Does not present any
1282 and 1268 (2 sharp bands)	1280
1083	Does not present any

[0005] Two other synthesis, affording azithromycin **1** as a form that should differ from the crystalline ones previously mentioned, have also been described. In these cases, azithromycin is obtained by simple evaporation to dryness. However, in these documents there is no reference to the crystalline statc of the azithromycin thus obtained.

Patent	Applicant (Author)	Priority	Procedure
• WO 94/26758 a) US 5,686,587 b) EP 0,699,207 c) ES 2,104,386	PFIZER (B.V. Yang)	May 19,1993	Methylene chloride evaporation
• BE 892,357 • US 4,517,359	PLIVA (S. Djokic)	Mar. 3, 1981	Chloroform evaporation

[0006] In the following table are summarized the different procedures for the preparation of both crystalline forms of azithromycin **1**.

Crystalline form	Patent	Applicant (Author)	Priority	Procedure
HYGROSCOPIC MONOHYDRATE	a) EP 0,101,186 b) US 4,474,768	PFIZER (G.M. Bright)	July 19, 1982	Recrystallization from ethanol/water
HYGROSCOPIC MONOHYDRATE	c) EP 0,298,650	PFIZER (D. Allen)	July 9, 1997	Recrystallization from ethanol/water
NON-HYGROSCOPIC DIHYDRATE	d) EP 0,298,650 e) WO 89/00576 f) ES 2,038,756	PFIZER (D. Allen)	July 9, 1997	Recrystallization from THF / petroleum ether/ water • Recrystallization from acetone/water • Recrystallization from other solvents (methanol, DMF, acetonitrile, dioxane, ...) and water
NON-HYGROSCOPIC DIHYDRATE	g) CN 1,093,370 (*Chem. Abs.* 29525q,124,**1996**)	Eaming Zhuanli.... (Q. Song)	Dec. 10, 1993	
NON-HYGROSCOPIC DIHYDRATE	h) EC 95-1389	CHEMO-TECNICA SINTYAL	May, 1995	Recrystallization from acetone/water

(continued)

Crystalline form	Patent	Applicant (Author)	Priority	Procedure
NON-HYGROSCOPIC DIHYDRATE	i) EP 0,827,965 j) ES 2,122,905 k) US 5,869,629	ASTUR PHARMA (M.Bayod)	July 11, 1996	Recrystallization from acetone/ water
NON-HYGROSCOPIC DIHYDRATE	l) EP 0,941,999	HOVIONE (W.Heggie)	Mar 13, 1998	Precipitation from a base neutralized acid solution of azithromycin in acetone/water
Crystalline form	**Article**	**Author**	**Date**	**Procedure**
NON-HYGROSCOPIC DIHYDRATE	· *J. Chem. Res.*, **1988**, 132 m) *idem miniprint.*, **1988**, 1239,	S.Djokic (PLIVA)	May, 1988 (received June 4, 1987)	Two recrystallizations: 1. Precipitation from a base neutralized acid solution of azithromycin in acetone/ water. 2. From ethyl ether.
NON-HYGROSCOPIC DIHYDRATE	· *J. Org. Chem*, **1997**, 62, (21), 7479-7481	M.Bayod (ASTUR-PHARMA)	Nov., 1997 (received May 1, 1997)	Recrystallization from acetone/water
HYGROSCOPIC MONOHYDRATE	· *J. Org. Chem*, **1997**, 62, (21), 7479-7481	M.Bayod (ASTUR PHARMA)	Nov., 1997 (received May 1, 1997)	Recrystallization from ethanol/water

DESCRIPTION OF THE INVENTION.

[0007] The present invention provides a series of new procedures for the preparation of azithromycin **1**:

- A procedure for the preparation of its crystalline dihydrate form, characterized by crystallization of azithromycin from a mixture of *tert*-butanol / water. In this procedure crystalline azithromycin monohydrate is dissolved in *tert*-butanol and, after water addition, is allowed to crystallize for a period of 48-72 hours.
- A procedure for the preparation of its crystalline dihydrate form, characterized by crystallization of azithromycin from a mixture of *tert*-butanol / petroleum ether / water. In this procedure, crystalline azithromycin monohydrate is dissolved in *tert*-butanol and added to a mixture of petroleum ether and water. This solution is allowed to crystallize for a period of 48-72 hours.

[0008] The procedures that are the object of the present invention are advantageous over previously described methods, essentially at industrial scale because these crystallization procedures, which are characterized by slow crystal growth, greatly improve the homogeneity and particle distribution of different batches. This minimizes the presence of the non-crystalline fraction (detected by X-Ray and DSC) that is always present in crystalline azithromycin dihydrate obtained by the methods reported in the literature and above cited.

[0009] Crystalline azithromycin dihydrate is unequivocally characterized by means of its IR (Fig. 2) and X-Ray Diffraction (Fig. 4) spectra and DSC thermogram (Fig. 3).

EXPERIMENTAL PART

• Preparation of 9-deoxo-9a-aza-11,12-desoxy-9a-homoerythromycin A 11,12-hydrogenorthoborate.

[0010] 89 g of 9-deoxo-6-desoxy-6,9-epoxy-9,9a-dihydro-9a-aza-homoerythromycin A are dissolved in 450 ml of methanol and cooled down between -5° and -10 °C. While keeping the temperature in the specified interval 16 portions of 2.2 g each of sodium borohydride are added. Temperature and stirring conditions are maintained for two additional

hours and the bulk of the reaction is allowed to reach 20 °C. After 20 h, the methanol is evaporated to dryness. The residue is dissolved in 500 ml of methylene chloride and 750 ml of water and shaked for 30 min. The organic phase is separated and the aqueous phase is extracted with 250 ml of methylene chloride. The organic phases are combined, filtered over celite, dried with anhydrous sodium sulphate and concentrated to dryness to yield 85 g of 9-deoxo-9a-aza-11,12-desoxy-9a-homoerythromycin A 11,12-hydrogenorthoborate.

IR (KBr)	ν_{max}= 3500, 2980, 2960, 1730, 1470, 1390, 1170, 1090, 1060 cm^{-1}
^{1}H-NMR ($CDCl_3$) (partial)	δ = 2.21 (NMe_2), 3.27 (OMe) ppm.
^{13}C-NMR ($CDCl_3$) (partial)	δ = 180.0 (C=O), 79.63 (C_{11}), 76.46 (C_{12}) 58.7 (C_{10}), 57.1 (C_9), 49.4 (OMe), 40.2 (NMe_2) ppm
^{11}B-NMR ($CDCl_3$)	δ = 9.9 ppm $\omega_{1/2}$= 200 Hz
TLC	rf= 0.28 (petroleum ether: ethyl acetate: diethylamine 75:25:10) developer: ethanol/vanillin (sulphuric acid)

• Preparation of 9-deoxo-9a-aza-11,12-desoxy-9a-methyl-9a-homo-erythromycin A 11,12-hydrogenorthoborate.

[0011] 50 g of 9-deoxo-9a-aza-11,12-desoxy-9a-homoerythromycin A 11,12-hydrogenorthoborate are dissolved in 500 ml of chloroform, and subsequently a mixture of 5.5 ml of formic acid and 11.75 ml of aqueous 35-40% formaldehyde is added. The reaction mixture is heated under pressure for 14 hours and subsequently cooled down to 15-20°C. 500 ml of water are added and the mixture is taken to pH=4 by adding 20% sulphuric acid. The mixture is shaken for 15 min and the lower organic layer is separated. The alkaline aqueous phase is extracted with 2x100 ml methylene chloride. The organic phases are combined and filtered over celite, dried with anhydrous sodium sulfate and evaporated to dryness. The residue obtained is washed twice with 250 ml of ethyl ether, yielding a dry residue of 29 g of 9-deoxo-9a-aza-11,12-desoxy-9a-methyl-9a-homo-erythromycin A 11,12-hydrogenorthoborate.

IR (KBr)	ν_{max}= 3500, 1730, 1470, 1390, 1090, 1070 cm^{-1}
^{1}H-NMR ($CDCl_3$) (partial)	δ = 2.00 (NMe_2), 2.30 (NMe), 3.37 (OMe) ppm
^{13}C-NMR($CDCl_3$) (partial)	δ= 179.9 (C=O), 79.40 (C_{11}), 77.09 (C_{12}), 68.84 (C_9), 64.08 (C_{10}), 49.36 (OMe), 40.18 (NMe_2), 34.39 (NMe) ppm
^{11}B-NMR($CDCl_3$)	δ= 10.1 ppm $\omega_{1/2}$= 180 Hz
m/e	M^+= 775.5
TLC	rf= 0.38 (petroleum ether : ethyl acetate : diethylamine 75:25:10) developer: ethanol/vanillin (sulphuric acid)

• Hydrolysis of 9-deoxo-9a-aza-11,12-desoxy-9a-methyl-9a-homo-erythromycin A 11,12-hydrogenorthoborate. Synthesis of 9-deoxo-9a-aza-9a-methyl-9a-homo-erythromycin A (Azithromycin).

[0012] 22 g of 9-deoxo-9a-aza-11,12-desoxy-9a-methyl-9a-homo-erythromycin A 11,12-hydrogenorthoborate are dissolved in 250 ml of acetonitrile to which 125 ml of water are subsequently added. 20% sulphuric acid is added to the mixture to take it to pH=2, and stirring is maintained for 30 min. The acidic solution is poured into a mixture of 350 ml of methylene chloride and 350 ml of water, inmediately adding 48% lime until pH=9. The mixture is shaken for 15 min and the lower organic phase is separated. The alkaline aqueous phase is extracted with 2x100 ml of methylene chloride. The combined organic phases are filtered over celite and evaporated to dryness. The residue is dissolved in 50 ml of ethanol and 60 ml of water are added over 30 min. Precipitation is allowed for 2 h, and the solid is collected by filtration and vacuum-dried at 40°C to yield 15 g of 9-deoxo-9a-aza-9a-methyl-9a-homo-erythromycin A (Azithromycin).

IR (KBr)	ν_{max}= 3500, 3000, 2970, 1740, 1470, 1380, 1280, 1060 cm^{-1}
^{1}H-NMR ($CDCl_3$) (partial)	δ = 2.31 (NMe_2), 2.34 (NMe), 3.38 (OMe) ppm
^{13}C-NMR ($CDCl_3$) (partial)	δ = 178.9 (C=O), 73.08 (C_{12}), 72.32 (C_{11}), 69.88 (C_9), 62.43 (C_{10}), 49.37 (OMe), 40.23 (NMe_2), 35.92 (NMe) ppm

(continued)

m/e	M^+= 749.5
HPLC	corresponds according to *USP XXIII*
TLC	rf= 0.62 (petroleum ether : ethyl acetate : diethylamine 75:25:10) developer: ethanol/vanillin (sulphuric acid)

• Preparation of 9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin A dihydrate. Method A.

[0013] 25 g of crystalline azithromycin monohydrate are dissolved in 130 ml of *tert*-butanol heating at 30°C. This solution is filtered and 130 ml of water are added over 6 h. The resulting mixture is taken to pH=11 by addition of NaOH 2N, cooled down below 10°C and subsequently stirred for 48-72 h. The crystals are collected by filtration and dried (80 mm Hg / 25°C) to yield 15 g of azithromycin dihydrate.

IR (KBr) ν_{max}= 3560, 3496, 1740, 1470, 1380, 1344, 1282, 1268, 1251, 1093 cm^{-1} ^{1}H-NMR ($CDCl_3$), ^{13}C-NMR ($CDCl_3$), m/e, TLC and HPLC are identical to those of the previous example.

• Preparation of 9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin A dihydrate. Method B.

[0014] 25 g of crystalline azithromycin monohydrate are dissolved in 50 ml of *tert*-butanol heating at 30°C. This solution is filtered and discharged over a mixture of 500 ml of petroleum ether and 20 ml of water. The resulting mixture is cooled down below 10°C and subsequently stirred for 48-72 h. The crystals are collected by filtration and dried (80 mm Hg / 25 °C) to yield 12 g of azithromycin dihydrate.
IR (KBr), ^{1}H-NMR ($CDCl_3$), ^{13}C-NMR ($CDCl_3$), m/e, TLC and HPLC are identical to those of the previous example.

Claims

1. A process for the preparation of 9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin A (Azithromycin) in its crystalline dihydrate form **characterized by** crystallization after water addition to a solution of azithromycin in *tert*-butanol.

2. A process for the preparation of 9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin A (Azithromycin) in its crystalline dihydrate form **characterized by** crystallization from a solution of azithromycin in *tert*-butanol by addition over a mixture of petroleum ether and water.

3. A process for the preparation of 9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin A (Azithromycin) in its crystalline dihydrate form **characterized by**:

   ✓ Hydrolysis of 9-deoxo-9a-aza-11,12-desoxy-9a-methyl-9a-homo-erythromycin A 11,12-hydrogenorthoborate in an organic solvent (ethyl acetate, acetonitrile, methanol or ethanol) by the action of a dilute acid (sulphuric acid, hydrochloric acid, oxalic acid) at room temperature and at a pH range comprised between 2 and 4.
   ✓ Dissolution of azithromycin in *tert*-butanol.
   ✓ Crystallization by addition of water.

4. A process for the preparation of 9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin A (Azithromycin) in its crystalline dihydrate form **characterized by**:

   ✓ Hydrolysis of 9-deoxo-9a-aza-11,12-desoxy-9a-methyl-9a-homo-erythromycin A 11,12-hydrogenorthoborate in an organic solvent (ethyl acetate, acetonitrile, methanol or ethanol) by the action of a dilute acid (sulphuric acid, hydrochloric acid, oxalic acid) at room temperature and at a pH range comprised between 2 and 4.
   ✓ Dissolution of azithromycin in *tert*-butanol.
   ✓ Crystallization by addition over a mixture of petroleum ether and water.

Figure 1. Synthesis of Azithromycin

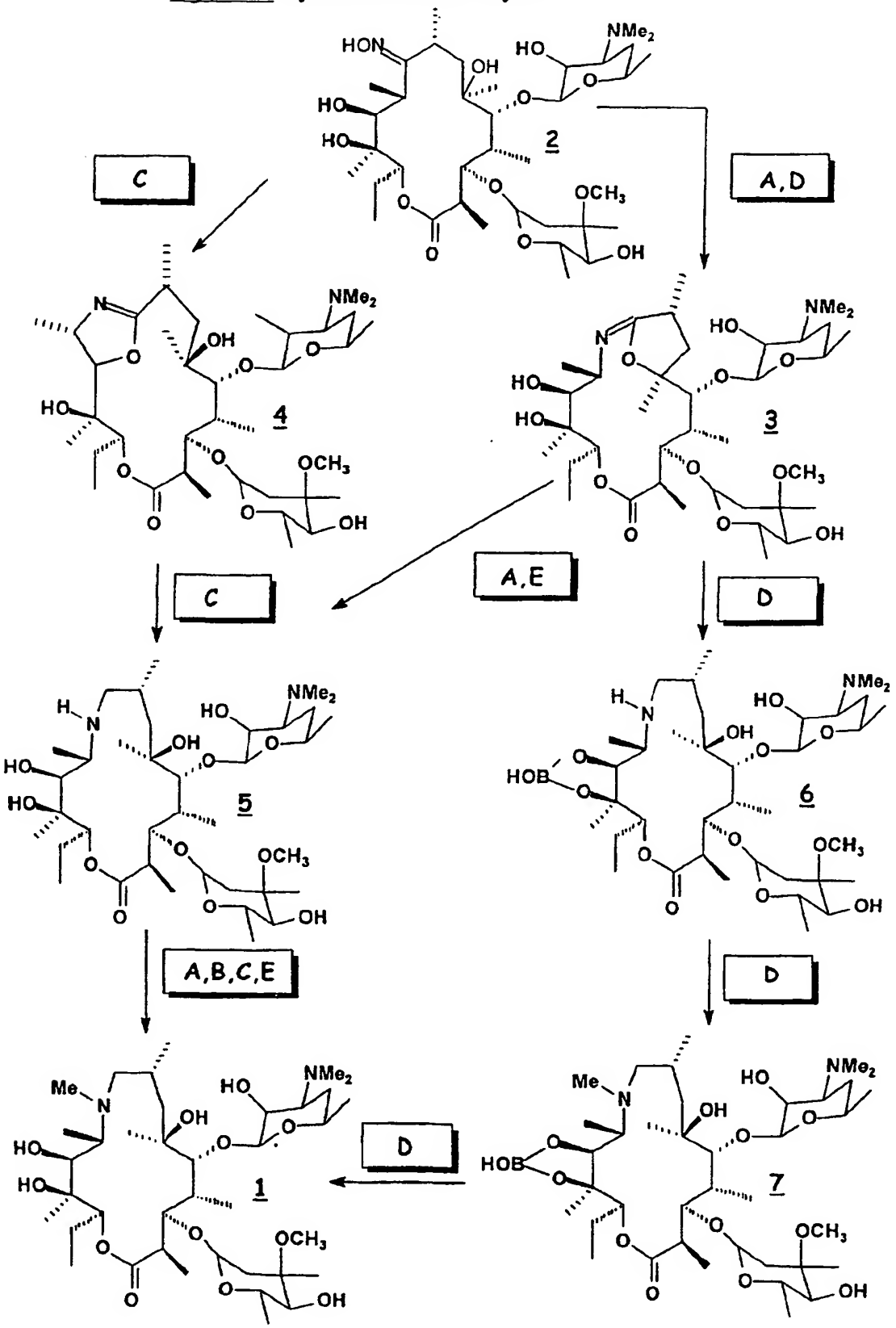


Figure 2

Crystalline Azithromycin Dihydrate

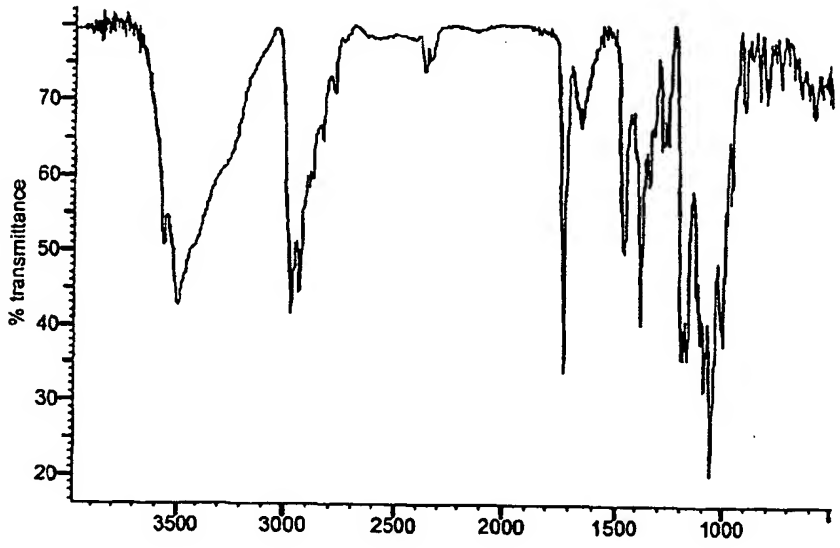


Figure 3

Thermogram of crystalline Azithromycin dihydrate

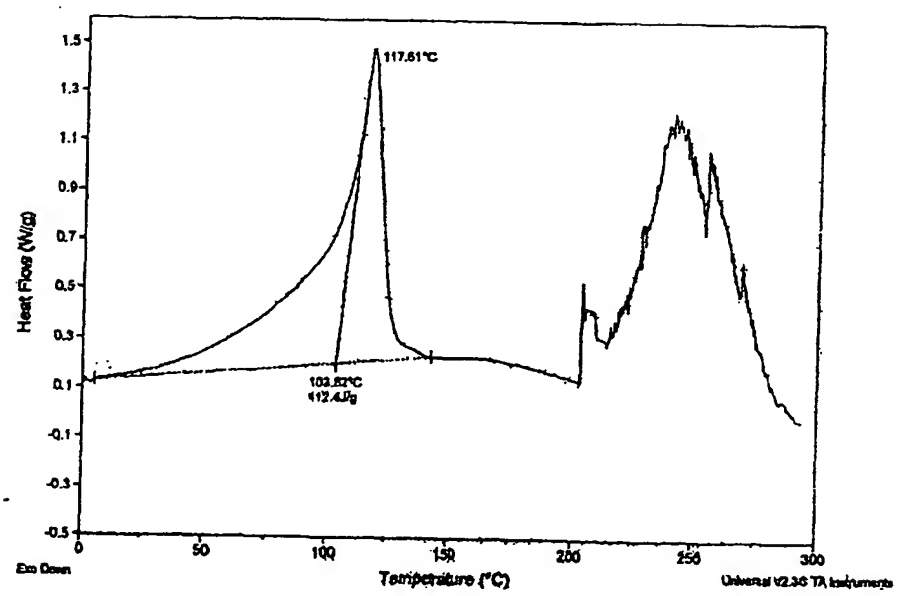
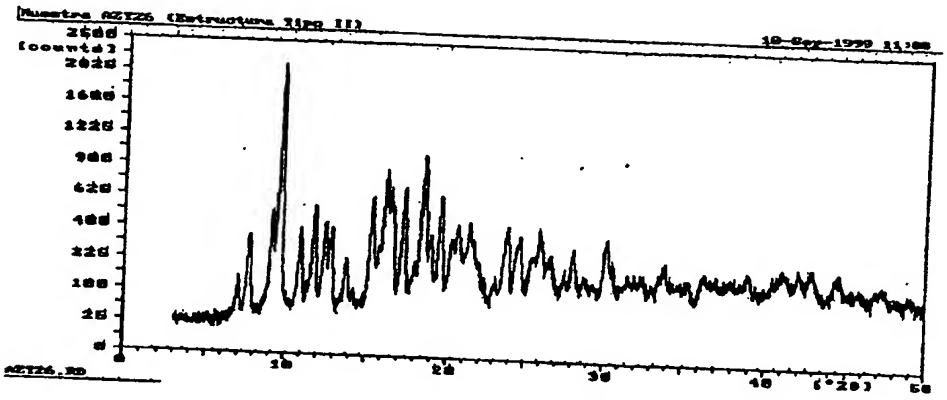


Figure 4

Crystalline Azithromycin dihydrate



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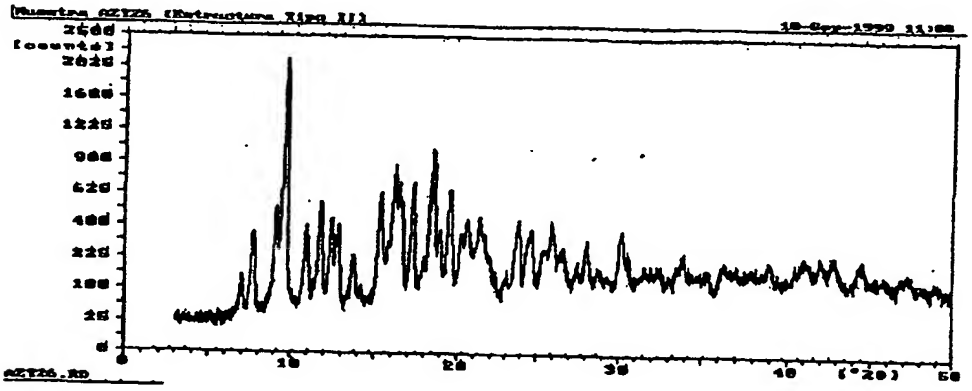

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EUROPEAN SEARCH REPORT

Application Number
EP 01 20 4550

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
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A	WO 99 58541 A (BIOCHEMIE SA ;DIAGO JOSE (ES); BOSCH IMMACULADA (ES); CENTELLAS VI) 18 November 1999 (1999-11-18) * page 11; example 2; table 1 *	1-4	

TECHNICAL FIELDS SEARCHED (Int.Cl.7)

C07H

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	14 January 2003	Scott, J

CATEGORY OF CITED DOCUMENTS

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